

## DESCRIPTION

## PHOSPHODIESTERASE 10A INHIBITOR

## 5 TECHNICAL FIELD

The present invention relates to a phosphodiesterase 10A inhibitor containing a pyrazolo[1,5-a]pyridine derivative as an active ingredient. The present invention also relates to therapeutic agents for treating Parkinson's disease,  
10 Huntington's disease, Alzheimer's disease, and schizophrenia that contain the phosphodiesterase 10A inhibitor as an active ingredient.

## BACKGROUND ART

15 [Patent Article 1] Japanese Patent Laid-Open Publication  
No. 2001-161379

[Patent Article 2] Japanese Patent Laid-Open Publication  
No. 2000-224992

[Patent Article 3] Pamphlet of International Patent  
20 Application No. WO 01/24781

[Patent Article 4] Japanese Patent Laid-Open Publication  
No. Sho 52-134870

[Patent Article 5] Japanese Patent Laid-Open Publication  
No. Hei 2-131424

25 [Non-patent Article 1] Fujishige et al., J. Biol. Chem.,

274:18438-45, 1999.

[Non-patent Article 2] Scott et al., Proc. Natl. Acad. Sci. USA, 96:7071-6. 1999 ; Fujishige et al., Eur. J. Biochem., 266:1118-27, 1999.

5 [Non-patent Article 3] Fujimoto et al., J. Neuroimmunol., 95:35-42, 1999.

[Non-patent Article 4] Souness et al., Brit. J. Pharmacol., 11:1081-8, 1994 ; Murashima et al., Jpn. Pharmacol. Ther., 26:41-5, 1998 ; Kishi et al., J. Cardiovasc. Pharmacol., 36:65-  
10 70, 2000.

Phosphodiesterase (which may be referred to simply as PDE, hereinafter) is an enzyme that degrades cyclic AMP (cAMP) and cyclic GMP (cGMP), which play a significant role in various reactions in cells. In response to various extracellular  
15 signals, cAMP and cGMP are generated from ATP and GTP by the action of adenylyl cyclase and guanylyl cyclase and are degraded by PDE into 5'-AMP and 5'-GMP, respectively. Eleven different families of PDE have been identified so far. Each family specifically degrades cAMP, cGMP or both and has a  
20 different tissue distribution. It is thus believed that different types of PDE control cellular reactions in different organs.

Among many PDE inhibitors that have thus far been put to practical application are those inhibiting PDE3 (treatment for  
25 heart failure), PDE4 (treatment for asthma and COPD) and PDE5

(treatment for male erectile dysfunction).

In 1999, the presence of PDE10A, a new subtype of PDE, was reported in humans, mice, and rats. PDE10A is involved in the regulation of intracellular cAMP and cGMP levels and, in humans, exists predominantly in brain, testis, and thyroid. In brain, PDE10A is predominantly expressed in putamen and caudate nucleus that form neostriatum (Non-patent Article 1). High level expression of PDE10A is observed in brain and testis of mice and rats (Non-patent Article 2).

Human PDE10A gene was isolated and was evaluated for the susceptibility to different PDE inhibitors. The results indicated that PDE10A was inhibited by dipyridamole (Patent Article 1 and Patent Article 2). No specific examples were presented of application of the compound to actual disorders.

Only one example was reported, in which minocycline was used in the patients with Huntington's disease as a PDE10A modulator with positive results (Patent Article 3).

Pyrazolo[1,5-a]pyridine derivatives as represented by the general formula (1) below are known (Patent Article 4). Of these derivatives, 3-isobutyryl-2-isopropyl pyrazolo[1,5-a]pyridine, commonly known as ibudilast, is widely used as a cerebral circulation improver or a treatment for bronchial asthma and allergic conjunctivitis. Known effects of ibudilast include enhancement of the relaxation effect of prostacyclin (PGI<sub>2</sub>) on cerebral vascular smooth muscle, enhancement of the

inhibitory effect of prostacyclin (PGI<sub>2</sub>) on platelet aggregation, suppression of airway contraction, suppression of leukotriene release and inhibition of PDE, as well as improvement of memory disorder (Patent Article 5) and amelioration of multiple sclerosis (Non-patent Article 3).

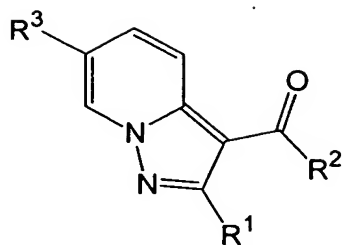
Although ibudilast has been described to inhibit PDE<sub>3</sub>, PDE<sub>4</sub>, and PDE<sub>5</sub> (Non-patent Article 4), nothing has been known about its activity as a PDE<sub>10</sub> inhibitor.

#### DISCLOSURE OF THE INVENTION

The aim of the present invention is to provide a PDE<sub>10A</sub> inhibitor, which is a potential prophylactic or therapeutic agent against Parkinson's disease, Huntington's disease, Alzheimer's disease or, schizophrenia.

In the course of our search for a potential cure for diseases such as Parkinson's disease, Huntington's disease, Alzheimer's disease, and schizophrenia, the present inventors have discovered that some of the compounds with pyrazolopyridine structure have an activity to inhibit PDE<sub>10A</sub>. This discovery ultimately led to the present invention.

Specifically, the present invention concerns a phosphodiesterase 10A inhibitor that contains as an active ingredient a pyrazolo[1,5-a]pyridine derivative represented by the following general formula (1):



(1)

wherein  $R^1$  and  $R^2$  are each independently a hydrogen atom or a lower alkyl group having 1 to 4 carbons; and  $R^3$  is a hydrogen atom, a lower alkyl group having 1 to 4 carbons, or a lower alkoxy group having 1 to 3 carbons. The present invention also concerns a therapeutic agent for treating Parkinson's disease, Huntington's disease, Alzheimer's disease, and schizophrenia that contains the above-described phosphodiesterase 10A inhibitor.

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#### BEST MODE FOR CARRYING OUT THE INVENTION

The present invention will now be described in detail.

Pyrazolo[1,5-a]pyridine derivatives as represented by the general formula (1) above are known compounds. Of these derivatives, 3-isobutyryl-2-isopropyl pyrazolo[1,5-a]pyridine, commonly known as ibudilast, is widely used as a cerebral circulation improver or a treatment for bronchial asthma and allergic conjunctivitis.

In the general formula (1), the lower alkyl group having 1 to 4 carbons may be methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, or tert-butyl and is preferably isopropyl. The lower alkoxy group having 1 to 3 carbons may be methoxy,

ethoxy, propoxy, or isopropoxy.

When examined for the ability to inhibit different PDEs using cAMP and cGMP as a substrate, the compound of the present invention represented by the general formula (1)

5 showed the strongest inhibition against PDE10A.

Parkinson's disease is known to be caused by a decreased supply of dopamine to neostriatum. It is believed that dopamine regulates the cAMP level in neostriatum (putamen and caudate nucleus) via D1 and D2 dopamine receptors present in  
10 the neostriatum. Since PDE10A is present specifically in these regions, an inhibitor of the enzyme is expected to have a potential as a novel treatment for Parkinson's disease.

Huntington's disease is characterized by the degeneration and shrinkage of neostriatum and its causative gene includes  
15 an abnormal extension of CAG repeats, suggesting inhibition of CREB-dependent transcription, an essential process in the survival of cells. Thus, a PDE10A inhibitor, which acts to increase the cAMP level in neostriatum, is expected to have a potential as a novel treatment for Huntington's disease.

20 Alzheimer's disease is a cerebral degenerative disease that leads to severe dementia and is characterized by deposition of beta-amyloids in nerve cells, degeneration of neurofibrils, loss of nerve cells and abnormal decrease of acetylcholine and other neurotransmitters. cGMP is known to  
25 facilitate the release of certain neurotransmitters, such as

glutamic acid and acetylcholine, facilitate the growth of dendrites, increase the viability of nerve cells, and suppress apoptosis of nerve cells induced by beta-amyloids. Thus, by increasing brain cGMP level, Alzheimer's disease and other cerebral degenerative disorders may be ameliorated. Therefore, a PDE10A inhibitor, which may cause an increase in the brain cGMP level, is expected to have a potential as a novel treatment for Alzheimer's disease.

A major cause of schizophrenia is believed to be an imbalance of signaling mediated by neurotransmitters, glutamic acid, serotonin, and dopamine. The most plausible hypothesis for the cause of schizophrenia seems to be excessive release of dopamine in neostriatum. cGMP is known as a second messenger that facilitates the release of glutamic acid in neostriatum, so that an elevated cGMP level may modulate the balance of signaling mediated by neurotransmitters in neostriatum. Therefore, a PDE10A inhibitor, which may cause an increase in the cGMP level in neostriatum, is expected to have a potential as a novel treatment for schizophrenia.

For the reasons described above, the pyrazolo[1,5-*a*]pyridine derivatives represented by the general formula (1), which have proven to be effective PDE10A inhibitors, are thought to be effective in the prevention and treatment of Parkinson's disease, Huntington's disease, Alzheimer's disease, and schizophrenia.

## Example

The present invention will now be described in detail with reference to examples, which are not intended to limit the scope of the invention in any way.

### <Example> Inhibition of PDEs by Ibudilast

#### 1) Method

cDNA of full length PDEs, specifically, PDE1A3, PDE2A3, PDE3A, PDE3B, PDE4A4, PDE4B2, PDE4C2, PDE4D3, PDE5A1, PDE5A2, PDE5A3, PDE7A2, PDE8A1, PDE9A2, PDE10A1, and PDE11A1, were isolated by RT-PCR from human RNA. The isolated cDNA fragments were introduced into Sf9 insect cells using Gateway system (Invitrogen) and Bac-to-Bac<sup>®</sup> Baculovirus Expression system (Invitrogen) to express PDE proteins. The recombinant proteins of PDE1A3, PDE2A3, PDE3A, PDE4A4, PDE4B2, PDE4C2, PDE4D3, PDE5A1, PDE5A2, PDE5A3, PDE7A2, PDE8A1, PDE9A2, PDE10A1, and PDE11A1 were purified by ion exchange chromatography from the supernatants or cell extracts of the Sf9 cells. For the recombinant PDE3B, the Sf9 cells expressing PDE3B protein at a high level were suspended in a RIPA buffer [150 mM NaCl, 10 mM Tris-HCl(pH8.3), 0.1% protease inhibitor cocktail (Product No.: P8849, Sigma)], and the whole suspension was used in the experiment described below.

A 4mM solution of ibudilast was serially diluted 4-fold



with a 15% DMSO solution to form ibudilast solutions of 15 nM to 4 mM (Final ibudilast concentrations used in the experiment were 1.5 nM to 400  $\mu$ M). 10  $\mu$ l of each ibudilast solution, 50  $\mu$ l of a [ $^3$ H]cAMP or [ $^3$ H]cGMP solution (diluted with a buffer (40 mM Tris-HCl (pH7.4), 10 mM MgCl<sub>2</sub>) to a concentration shown in Table 1), and 40  $\mu$ l of each of the recombinant human PDE proteins (in units shown in Table 1) were added to a 96-well plate and the mixtures were incubated at 30°C. for 20 min and then at 65°C. for 2 min. 25  $\mu$ l of 1 mg/ml 5'-nucleotidase (Crotalus atrox venom, Sigma) was then added to each well and the reactions were carried out at 30°C. for 10 min. Upon completion of the reaction, 200  $\mu$ l Dowex solution (300 mg/ml Dowex 1x8-400 (Sigma Aldrich), 33% Ethanol) was added and the mixtures were agitated at 4°C. for 20 min. 200  $\mu$ l MicroScint20 (Packard) was then added and the mixtures were analyzed by a scintillation counter (Topcount, Packard). IC50 values were determined by GraphPad Prism v.3.03 (GraphPad Software). The results are shown in Table 2.

**Table 1**

Enzyme (amount)	Substrate	Conc. substrate
PDE 1A3 ( $2 \times 10^{-6}$ units*)	cAMP	$2 \mu\text{M}$
PDE 1A3 ( $2 \times 10^{-6}$ units)	cGMP	$2 \mu\text{M}$
PDE 2A3 ( $2 \times 10^{-6}$ units)	cAMP	$2 \mu\text{M}$
PDE 3 (A11) ( $2 \times 10^{-6}$ units)	cAMP	$2 \mu\text{M}$
PDE 4 (A11) ( $2 \times 10^{-6}$ units)	cAMP	$2 \mu\text{M}$
PDE 5 (A11) ( $2 \times 10^{-6}$ units)	cGMP	$2 \mu\text{M}$
PDE 7A2 ( $2 \times 10^{-7}$ units)	cAMP	$0.2 \mu\text{M}$
PDE 8A1 ( $2 \times 10^{-7}$ units)	cAMP	$0.2 \mu\text{M}$
PDE 9A2 ( $2 \times 10^{-7}$ units)	cGMP	$0.2 \mu\text{M}$
PDE 10A1 ( $2 \times 10^{-7}$ units)	cAMP	$0.2 \mu\text{M}$
PDE 10A1 ( $2 \times 10^{-6}$ units)	cGMP	$2 \mu\text{M}$
PDE 11A1 ( $2 \times 10^{-6}$ units)	cAMP	$2 \mu\text{M}$
PDE 11A1 ( $2 \times 10^{-7}$ units)	cGMP	$0.2 \mu\text{M}$

\* 1 unit of PDE corresponds to an amount of the enzyme that hydrolyzes  $1 \mu\text{M}$  cAMP or cGMP at  $30^\circ\text{C}$  and pH7.5 in one minute.

## 5 2) Results

As shown in Table 2, the *in vitro* experiment using recombinant human PDE demonstrated that ibudilast had a particularly strong inhibition of PDE10A1.

10 **Table 2**

PDE	Substrate	IC <sub>50</sub> ( $\mu\text{M}$ )	PDE	Substrate	IC <sub>50</sub> ( $\mu\text{M}$ )
PDE 1A3	cAMP	40	PDE 5A1	cGMP	>400
PDE 1A3	cGMP	121	PDE 5A2	cGMP	88
PDE 2A3	cAMP	78	PDE 5A3	cGMP	127
PDE 3A	cAMP	124	PDE 7A2	cAMP	115
PDE 3B	cAMP	266	PDE 8A1	cAMP	49
PDE 4A4	cAMP	6	PDE 9A2	cGMP	>400
PDE 4B2	cAMP	6	PDE 10A1	cAMP	3
PDE 4C2	cAMP	11	PDE 10A1	cGMP	1
PDE 4D3	cAMP	6	PDE 11A1	cAMP	17
			PDE 11A1	cGMP	36

## INDUSTRIAL APPLICABILITY

As set forth, pyrazolo[1,5-a]pyridine derivatives

represented by the general formula (1) have proven to be strong inhibitors of PDE10A and have thus proven to be useful in the prevention and treatment of Parkinson's disease, Huntington's disease, Alzheimer's disease, and schizophrenia.